Nutritional Performance Factors in Collegiate Female Swimmers

A Senior Honors Thesis

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Abstract

Background: Iron deficiency (ID) is one of the most common nutrient deficiencies in the US, especially for female athletes. Iron status is negatively impacted by intense physical exercise by reducing serum iron levels through various proposed mechanisms. This can impair oxygen transport, which can decrease endurance capacity and energy levels, increase fatigue levels, and ultimately hinder aerobic exercise performance.

Purpose: The necessary level of serum ferritin to support high-level performance remains controversial and is poorly validated using field data. Ferritin levels of the Ohio State University Women's Swimming team has shown great variability over the past years when measured clinically. Therefore, the purpose of this study was to look at iron status and its association with performance in NCAA Division I female swimmers over a competitive season.

Methods: The study employed a pre, mid, and post-test design. Data collection started in September, and was collected roughly every three months. Serum ferritins were measured from a venous blood sample. Diets were estimated by the on-line Vioscreen Food Frequency tool to approximate daily consumption. The Australian Institute of Sport's athlete fatigue questionnaire was the primary self-report tool of fatigue symptoms. Performance was gauged by mid- and NCAA swim-event times.

Results: Serum ferritins demonstrated wide variability across the season. A repeated measures ANOVA showed an increased ferritin at the end of the season compared with first and second measures. There were no meaningful relationships between change in ferritin and performance, but there were correlations with energy intake and fatigue levels at different points in the season.

Conclusion: This pilot study showed that ferritin levels changed throughout the year, but that performance and iron status (ferritin) were not correlated. However, future research should look further into reasons for why ferritin levels changed.

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Introduction:

Iron deficiency is one of the most common nutrient deficiencies in the US, and females are at a higher risk than males[19, 32]. Iron status is negatively impacted by intense physical exercise by reducing serum iron levels through sweating, changes in saturation levels of transferrin (the protein that binds and carries iron through the body) and inhibition of the release of iron from its carrier, as well as a decrease in iron-containing oxidative enzymes [8]. As a result, this can impair oxygen transport, which can decrease endurance capacity and energy levels, increase fatigue levels, and ultimately hinder aerobic exercise performance [4]. The necessary level of serum ferritin to support high-level performance remains controversial and is poorly validated using field data. Ferritin levels of the Ohio State University Women's Swimming team has shown great variability over the past years when measured clinically. Therefore, the purpose of this study is to evaluate the relationship of the following:

1. How does serum ferritin change over a competitive swimming season? Do supplementation and diet relate to this change?
2. Is there a relationship between serum ferritin and performance, and does this differ by training group?

Literature Review:
The majority of the body's iron is stored in the liver, spleen and bone marrow cells. While there are several methods to measure total body iron stores, the preferred and most commonly used method for athletic performance is by means of measuring plasma ferritin levels. Ferritin reflects total body iron levels because it is the major iron-storage compound: as the iron status of the body increases, the production of ferritin also increases proportionally [16]. Measuring the storage protein (ferritin) is considered proactive in an attempt to recognize low iron status before iron deficiency anemia (low hemoglobin). In terms of measuring iron status, serum ferritin levels are the most widely used indicators of total body iron levels.

Iron is an important nutrient required by the body for both physical and cognitive functions [2]. Iron deficiency (ID) is the most common nutrient deficiency in the United States, and is the imbalance of iron absorption and excretion in the body [19], causing iron levels to become depleted [4]. Iron absorption would obviously be impacted by iron consumed via the diet, but it is also controlled by the enterocytes of the small intestine, where the majority of the nutrient is absorbed [35].

Females, especially adolescents and those who are of childbearing age, are more susceptible to ID than their male counterpart due to the increased blood loss through the menstrual cycle and potential lower dietary consumption levels. Iron loss increases substantially during the menstrual cycle, especially if the cycle is heavy or lasts longer than five days [32]. On average, women lose 1-2.5 mg of iron per day during menstruation [24]. Currently, about 12% of women ages 20-49 are affected by low iron status [2]. Female athletes, specifically aerobically-based athletes, are even further susceptible to other means of iron loss such as gastrointestinal bleeding, sweating, and through intense physical exercise [8]. It has been found that intense physical exercise negatively effects iron status by reducing serum iron levels through several mechanisms including: changes in saturation levels of transferrin (the protein that binds and carries iron through the body), inhibition of the release of iron from its carrier (transferrin), as well as a decrease in iron-containing oxidative enzymes. As a result, this can impair blood-oxygen transport, which can decrease endurance capacity and energy levels, increase fatigue levels, and ultimately hinder aerobic exercise performance [8].

Iron deficiency is defined as too little iron in the blood [16]. However, if this condition persists over time, iron deficiency anemia (IDA) can develop, which is when iron levels in the body reach a low enough level where synthesis of red blood cells is impaired. Red blood cells carry oxygen throughout the body, and are constantly being created and destroyed. When the body does not produce enough red blood cells, the overall concentration of red blood cells decreases. This in turn negatively affects the body's ability to deliver oxygen to the necessary muscles and tissues that help the body function, resulting in decreased cognitive and physical function [17].

Disorder	Serum Ferritin Level	Hemoglobin Level
Iron Deficiency without Anemia	<15 mg/dL	>13.5 g/dL
Iron Deficiency with Anemia	<10 mg/dL	11.7 g/dL - 13.5 g/dL
Anemia	<10 mg/dL	<11.7 g/dL

[9, 17, 18, 30]

Iron is found in different parts of the body, with there being two different pools of iron – functional and storage. Functional iron is the type of iron that is used in the transporting of oxygen to various tissues and muscles in the body in order to produce energy, and this type of iron is found in hemoglobin, myoglobin and other iron-dependent enzymes. Iron that is bound to macrophages and hepatocytes represents the pool of storage iron [1]. Functional iron deficiency occurs when the iron status (storage iron) is adequate, but the iron is unable to be mobilized for erythropoiesis, and is thus functionally unavailable [33]. Absolute iron deficiency is when there is an iron-depleted storage pool [14]. Depleted iron status as found in ID leads to a reduction in tissue oxidative capacity, which in turn negatively affects endurance and energy levels. Oxygen carrying capacity is especially impaired when iron levels reach anemic levels with low hemoglobin [4]. As a result, female endurance-based athletes are at a greater risk for developing ID [32]. However, the question becomes, what is the level of insufficient iron that influences iron through these mechanisms? Also, is there a range of iron deficiency that is considered to be detrimental to performance for aerobically based female athletes, and if so, what is that range?

Iron supplementation to correct low ferritins (as opposed to waiting for IDA) is a current hot topic in sports nutrition. There are many forms of iron for oral supplementation. A 2013 meta-analysis of 111 iron supplementation studies determined that the ferrous sulfate supplement was an effective in iron repletion and was the best tolerated of the various oral iron supplements [7]. Recent literature has shown that intravenous (IV) iron supplementation has been successful in restoring mobilization of functional iron due to altering the way iron is released from storage [14, 33]. The IV treatment of athletes is currently controversial and deserves more research to ensure safety and lack of negative or unintended outcomes.

McClung et al [23] studied the effects of iron supplementation on aerobic capacity and maximal oxygen uptake for female soldiers affected with iron deficiency anemia, defined as a hemoglobin concentration lower than 10 g/dL. This study found that 8-weeks of daily ferrous sulfate supplementation had a positive effect on 2-mile run times, which were also related to maximal oxygen uptake. Supplementation also had positive effects on increasing transferrin and ferritin levels. However, studies regarding iron deficiency without anemia have had ambiguous results regarding improved aerobic capacity and performance as it relates to increasing iron status for deficient levels that are not low enough to be considered anemic [2].

There have been many studies conducted regarding the effects of iron deficiency, especially in athletes engaged in aerobic sports. The 2012 study conducted by Della Valle and Haas [10] looked at the effects of iron depletion without anemia on performance in female collegiate rowers. This study measured hemoglobin, serum ferritin, and soluble transferrin receptor levels in order to gauge iron status and rule out those that were anemic. Training volumes and intensities were recorded, along with performance measures through their best time in a 2-km race. It was found that serum ferritin levels were significantly related to performance: those identified as iron deficient through low serum ferritin levels (<20 mg/dL) had significantly slower 2-km times [9, 10]. Reinke et al. [31] studied the effects of different training levels on iron status in rowers, as well as in professional soccer players. Although these athletes were males, it was determined that 72% of them showed signs of iron deficiency in terms of mean corpuscle hemoglobin levels at the end of their championship seasons (after periods of intense training). Performance measures were not evaluated in terms of iron status, but it was found that short recovery periods, defined as three to four weeks of advised no physical activity, were insufficient in raising iron levels [31].

A 2007 study by Hinton [15] looked at various types of endurance athletes (including cross country runners, swimmers, rowers, etc.) that were both male and female that had low serum ferritin levels, but were not anemic according to hemoglobin. Participants were randomly assigned to a group that was supplemented with 30 mg ferrous sulfate daily or a control group without any supplementation. After 4-weeks, it was found that those in the control group had an earlier onset of CO_2 production during exercise, indicating an increased lactate production thus decreased aerobic performance. There was no statistically significant increase in performance for either group. However, the supplementation group maintained their original ventilatory threshold values, whereas the control group saw an attenuation of these values indicating a decrease in aerobic performance. This study also demonstrated that iron supplementation had an effect on performance and ferritin levels. In a similar to study to Hinton's, a 2001 study by Friedmann et al. [13] found that after 12-weeks of iron supplementation (100 mg of ferrous-glycine-sulfate twice daily), those who were in the supplementation group had iron repletion and an increased time to exhaustion on the maximal oxygen uptake test (performance marker) compared to those who were not supplementing. Literature suggests that iron supplementation of low ferritin athletes who are not yet anemic is supportive of aerobic performance.

There are studies specifically regarding the iron status of swimmers, but few have adequately demonstrated the potential influence on performance. The 2006 study by Petersen et al. [29] evaluated how training levels of collegiate level female swimmers influenced body composition, dietary intake and iron status. Hemoglobin, hematocrit and serum ferritin levels were measured. Although the hemoglobin and hematocrit levels improved over the course of the season, serum ferritin levels, and therefore iron status, did not change a significant amount. Those that started the study in an iron-depleted state (serum ferritin <12 mg/dL) did not improve levels, and there was found to be no correlation between iron status and performance [29]. A study of 42 Greek age-group (12-17 years old) swimmers (21 male, 21 female) was conducted by Tsalis [34] in order to determine if iron status changes during a season, and how it is affected by increasing daily iron intake

by measuring diet, iron status and performance. This study had three groups that either supplemented with iron, increased daily iron intake through diet, or did not change the diet/supplement. However, results were not evaluated with performance, and although the changes were not significant, serum ferritin levels tended to decrease throughout the season despite the higher intake of iron for two of the three groups [34]. Braun et al [3] measured iron status in a sample of ten collegiate swimmers compared to those of sedentary individuals with regards to immune function. However, this study did not measure the effect of iron status on performance or the potential influence of training levels [3]. Iron status has been popular to assess in swimmers, but the relationships to diet, supplementation, fatigue and performance have been inconsistently evaluated.

The purpose of this study is to evaluate and compare iron status as indicated by serum ferritin levels among female, NCAA Division I swimmers throughout a competitive season in relation to their performance, dietary intake, and training volumes. The working hypotheses are as follows:

1. Ferritin values will change throughout the course of the competitive season, increasing with increased dietary intake and/or supplementation.
2. Higher serum ferritin values will positively correlate to improved performance, regardless of training group.

Methods:

Subjects and Procedure

The longitudinal study was conducted on the women's varsity swim team at the Ohio State University for the 2013-2014 collegiate season. Participation was voluntary and there were 33 athletes invited to participate. This number dropped to 24 as 8 people left the team, and one dropped out of the study after the second measurement period, making the final participant number 23.

33 invited to participate

8 left the team → 24 participants remain

1 participant dropped out of study → 23 participants finish the study

A pre-, mid-, and post-test design was in place, with each data collection period taking place about three months apart. The first data collection coincided with the beginning of the season in September/October, the second collection correlating to the mid-season (late December), and the third collection occurring at the end of the season early in March. All procedures were approved by the Institutional Review Board, and subjects were consented prior to the start of the study (IRB# 2013H0231).

Iron Status

Venous blood draws were taken at each time point for analysis of serum ferritin levels (SF). These draws occurred at the Martha Morehouse Sports Medicine Pavilion nearby campus, and were taken by trained nurses and medical staff. Normal ferritin values were established by the team physician prior to the beginning of the study. Any participant below 30 mg/dL was recommended by the team dietitian to adopt eating patterns to boost intake of dietary iron (education sheet included in Appendix 1). Any participant below 20 mg/dL was sent to see the team physician for further testing and an oral iron supplementation of 325 mg ferrous sulfate daily (65 mg elemental iron). Iron supplementation was available to any participant who wanted it, and was monitored by sport staff, along with a concurrent Vitamin C oral supplement.

Nutritional Status

The Vioscreen Food Frequency online questionnaire was completed by the athletes at the data collection time points, and provided analysis of dietary intake of the past 90 days, including dietary iron levels [21]. The swimmers were given a username and password and were given a span of a week to take the online questionnaire on their own time. The Vioscreen tool was recently validated by Kristal et al. [21] and provided data supporting the use and reliability of online food questionnaires compared to paper questionnaires [21]. The

software allows for download of the nutritional markers for the athletes, and calories and dietary iron were of interest for this protocol.

Body Composition
The GE Lunar iDXA was used to analyze body composition yielding estimates of lean mass, body fat and bone mass for each athlete. This was measured during all three data collection periods. The iDXA scanner is a dual-energy X-ray absorptiometer used to measure lean mass and fat mass in many populations of athletes including elite athletes at the US Olympic Training Center. Lean body mass (LBM) and percent body fat (BF) were recorded and analyzed for this study.

Questionnaire to document fatigue and diet/supplementation
The Australian Institute of Sport's athlete fatigue questionnaire is the primary self-report tool of fatigue symptoms [22]. This questionnaire was given to the swimmers at each data collection time simultaneous to the blood draws. They were instructed not to discuss the questions amongst each other while filling it out. The second and third measurement points also included self-report questions about the supplementation protocol or diet changes the athlete felt she incorporated into her training plan (Appendix 2) and were not part of the Australian tool. For dietary modification options, subjects were presented five different potential dietary modifications to increase iron intake and asked to indicate all that they had incorporated. The number, type, and frequency of supplementation was also recorded.

Training Volume
Training volumes were recorded on a weekly basis, in terms of yards/meters swum per week. There were two training groups throughout the season – a distance group (D) and a sprint group (S). The distance group consisted of swimmers who swam longer events, and therefore had larger training volumes compared to the sprint group. The sprint group consisted of swimmers who swam shorter events and who were more anaerobically driven. The different training groups were taken into account, and each swimmer's group was recorded.

Performance
Performance (Perf) was recorded during the second and third measurement periods. The Ohio State Invite (OSU) swim meet occurred in the end of November and was the second period measurement, and the Big Ten Swimming and Diving Championships (BT) occurred at the end of February and was the third period measurement. Swimmers competed in two to three events, and their times were recorded for each of their events. Performance indicators were reported in terms of percent change, with a negative percent change indicating a drop in time and thus a performance improvement. The following percent change formula was applied:

$$((\text{Time}_{BT} - \text{Time}_{OSU})/ \text{Time}_{OSU}) \times 100 = \text{percent change}$$

This formula was applied for every race. Total percent change in performance for each athlete was calculated by determining the average percent change for all of their events.

Aerobic Capacity/Fitness

Fitness levels of the swimmers were measured at all three data collection periods using the Microfit Bike Test. This was a series of five tests measuring resting heart rate/blood pressure, weight, aerobic fitness, muscular strength, and back flexibility. All values were recorded in the computer system. The resting heart rate and blood pressure were measured using a computer-automated cuff in the sitting position. Weight was measured using the same scale for each person, each time. Aerobic fitness levels were measured via VO_2 levels through a two-minute staged, submaximal bike test using the Astrand protocol. A target heart rate was established at 115 beats per minute. Heart rate monitors were worn around the chest and were used to measure their heart rate. Once the swimmers reached two stages with heart rates successfully above the target, the test was terminated. VO2 values were calculated through extrapolation of the data with each individual's age-predicted maximum heart rate. Muscular strength was measured using an isometric force plate and bar. The swimmers would step on a force plate, pull up on a bar attached to the plate, and hold for three seconds. Elbows were maintained at 90 degrees and there was no back bending allowed. The last test was a sit-and-reach flexibility test. The swimmers placed their feet on the pedals and then reached forward and held the furthest number they could for three seconds, without bending their knees (this was also computer automated). This series of tests were all measured at least 12 hours after training in order to make sure resting heart rates were not elevated.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS version 21) was used for statistical analysis of the data. A repeated measures ANOVA was run to measure changes in ferritin across time (Table 3). To ensure the data met the underlying assumptions of the ANOVA, Mauchly's test of sphericity was used to determine if the sample presented equal variances at each time point where a p-value of <0.05 indicated the need to use the Greenhouse-Geisser model to account for the differences. (This non-parametric procedure compares the ranks of all of the results instead of comparing the results of the group as a whole.) Due to a low number of subjects, Spearman's correlations were run for correlations between ferritin changes, fatigue levels, supplementation compliance, dietary modifications, performance change, lean body mass change, percent body fat changes and caloric intake for the first and second half of the season. When comparing the distance and sprint groups for differences in performance variables, Levene's test of equality of variances was evaluated to ensure use of appropriate t-test values.

Results:

Descriptive Statistics

Descriptive statistics for the athletes in this study are reported in Table 1

Table 1 Descriptive Statistics for study cohort

	Mean (SD)	Range
Age	19.3 (1.34)	18-22
Height (in)	67.8 (2.20)	63.0-72.0
Weight (lbs)	150.6 (14.27)	122.0-191.0
BMI	23.0 (1.80)	20.1-27.7

* SD = Standard Deviation

Hypothesis 1: Ferritin values will change throughout the course of the competitive season, increasing with increased dietary intake and/or supplementation.

A repeated measures ANOVA was used to evaluate the changes in ferritin across the pre-season to mid-season and post-season measures. Model results are reported in Table 2 demonstrating a significant model (p=0.000). The Bonferroni adjustment was used to determine where in time the differences occurred. There was no significant difference between the SF levels between measurement between pre- and mid-season, but that there was a significant change of SF for the post-season measure when compared with pre- and mid-season measures (p=0.001, p=0.000 respectively). While the statistical analysis failed to reach significance for the early season differences, it is likely the influence of wide variability preventing those differences. The change in ferritin across the time points is plotted for each individual to demonstrate the variability (Figure 1).

Table 2: Repeated measures ANOVA of Ferritin across the season

Pairwise Comparisons

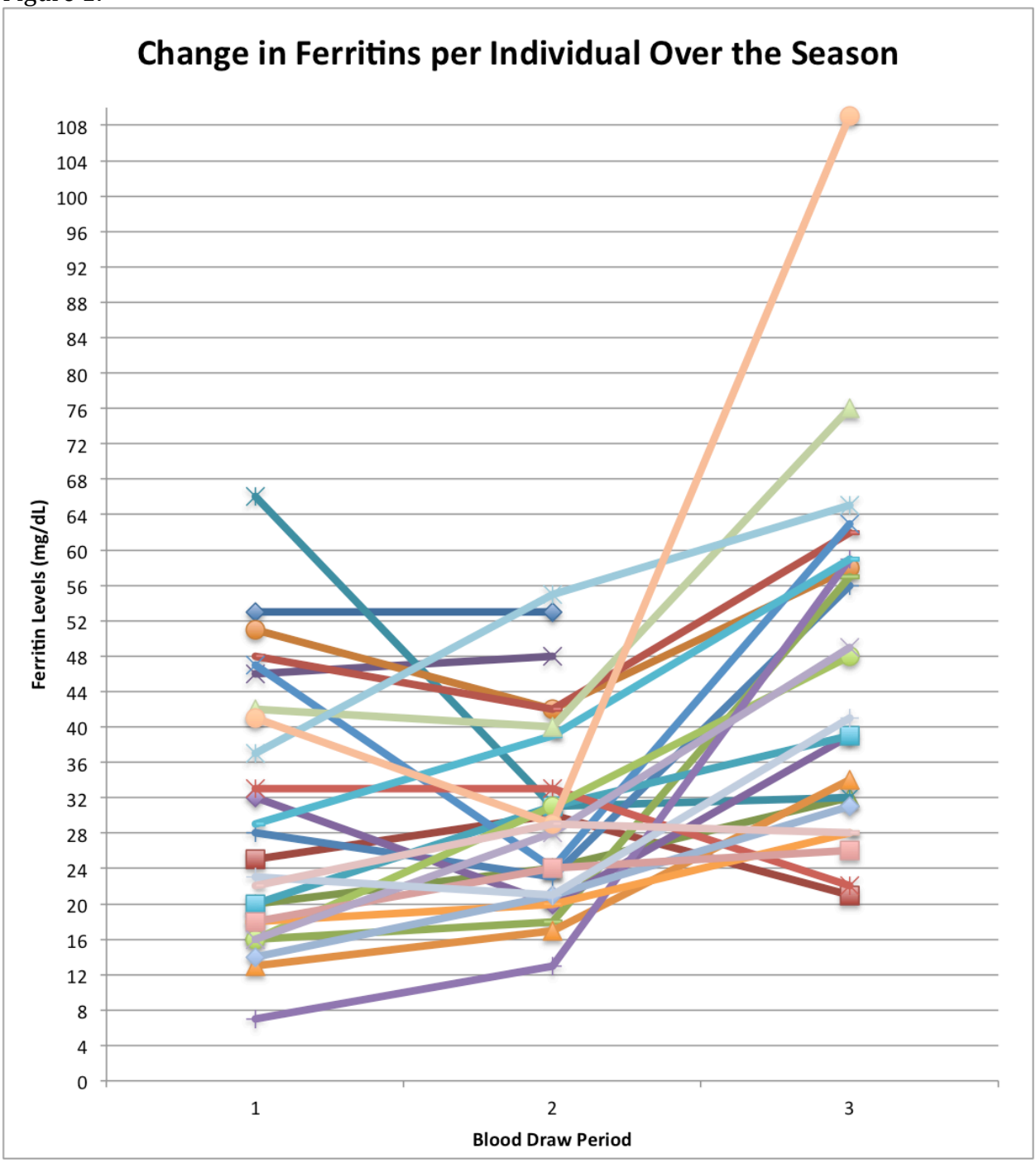
(I) ferritin	(J) ferritin	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-.125	2.460	1.000	-6.477	6.227
	3	-18.833*	4.221	.001	-29.732	-7.934
2	1	.125	2.460	1.000	-6.227	6.477
	3	-18.708*	4.033	.000	-29.122	-8.295
3	1	18.833*	4.221	.001	7.934	29.732
	2	18.708*	4.033	.000	8.295	29.122

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Figure 1:



Spearman correlations were used to determine the potential relationship of supplementation compliance and dietary modifications (to increase iron intake) on SF levels for the first and second halves of the season. The results from pre-season to mid-season (Table 3) are not statistically significant for either supplementation compliance or

dietary modifications (p=0.193, p=0.198 respectively). However, the weak correlations for both suggest that both supplementing and dietary modifications have a positive effect regarding improved SF levels (r_{supp}=0.275, r_{diet}=0.266). Results from mid-season to post-season also show similar results (Table 4). The FFQ was supposed to take 20-30 minutes to complete, but many athletes took it in less than 10 minutes, providing potentially unreliable caloric data.

Table 3: Relationship of ferritin changes during the first half of season

Correlations

Change in:			Ferritin	Dietary Modifications 2	Supplement Compliance 2	Fatigue 2
Spearman's rho	Ferritin	r	1.000	.266	.275	-.370
		Sig. (2-tailed)	.	.198	.193	.069
		N	25	25	24	25
	Dietary Modifications 2	r		1.000	.117	-.170
		Sig. (2-tailed)		.	.587	.418
		N		25	24	25
	Supplement Compliance 2	r			1.000	-.014
		Sig. (2-tailed)			.	.949
		N			24	24
	Fatigue 2	r				1.000
		Sig. (2-tailed)				.
		N				25

Table 4: Relationship of ferritin changes during the second half of season

Correlations

Change in:		Ferritin	Supplement Compliance 3	Dietary Modifications 3	Performance	Fatigue 3
Ferritin	Spearman r	1.000	.217	.168	-.083	.031
	Sig. (2-tailed)	.	.359	.454	.707	.887
	N	24	20	22	23	23
Supplemental Compliance 3	Spearman r		1.000	-.035	.016	-.171
	Sig. (2-tailed)		.	.881	.945	.472
	N		21	21	21	20
Dietary Modifications 3	Spearman r			1.000	.035	.258
	Sig. (2-tailed)			.	.875	.247
	N			23	23	22
Performance	Spearman r				1.000	-.323
	Sig. (2-tailed)				.	.132
	N				24	23
Fatigue 3	Spearman r					1.000
	Sig. (2-tailed)					.
	N					24

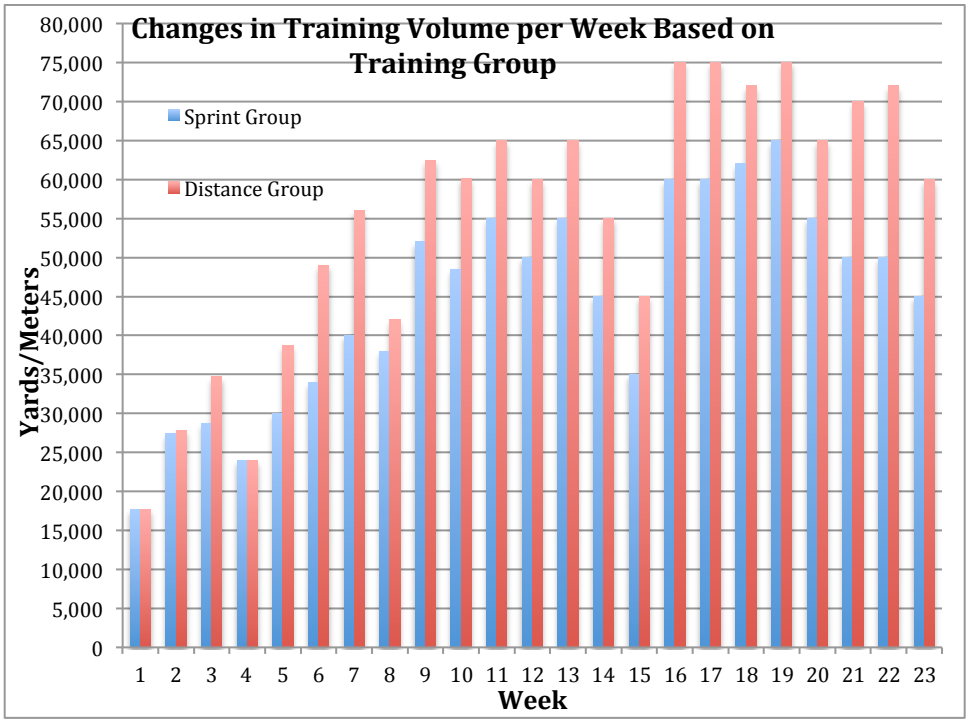
Hypothesis 2: Higher serum ferritin values will positively correlate to improved performance.

Spearman correlations were used to determine the relationship between change in ferritin and change in performance (Table 4). The results demonstrated that there was no significant relationship between improving SF and performance ($p=0.707$, $r=-0.083$). This refutes the original hypothesis. There were no differences found in performance between the two training volume groups (sprint and distance) (Table 5). The only statistical difference between the two groups was a trend towards a greater change in lean body mass in the distance athletes ($p=0.096$, <0.20).

Table 5: Comparisons by training volume groups for last half of season

Group		N	Mean	Std. Deviation	Std. Error Mean
Ferritin Change	Sprint	18	17.2778	21.19602	4.99595
	Distance	6	23.0000	15.45316	6.30872
Performance Change	Sprint	17	-.3541	1.58356	.38407
	Distance	8	-.2463	1.22400	.43275
% Body Fat Change	Sprint	18	1.3926	1.42536	.33596
	Distance	7	.3688	.99125	.37466
Lean Body Mass Change	Sprint	18	-1.3800	3.31109	.78043
	Distance	7	1.1014	1.34705	.50914

Figure 2: Changes in Training Volume per Week Based on Training Group



Other results of interest

Although the original hypotheses did not intend to look for these measures, there were significant correlations between LBM:%BF, performance:caloric intake, and ferritin:caloric intake (Table 6). Results suggested there was a moderate correlation between LBM and %BF ($p=0.005$, $r=-0.543$). This is statistically significant and suggests that as LBM increases, %BF decreases. The correlation of performance to caloric intake was also statistically significant ($p=0.038$), and had a weak, positive relationship ($r=-.466$). A negative sign indicates a drop in time suggesting a performance improvement as the daily caloric intake increased. A statistically significant relationship between ferritin changes

and caloric intake was also identified (p=0.021), indicating a moderate relationship between ferritin levels and caloric intake (r=0.511). This indicates that as caloric intake increases, ferritin levels also increase. Although the results were not statistically significant (p=0.069), a weak correlation was identified between changes in ferritin and fatigue levels. As ferritin levels increased over the first half of the season, fatigue levels decreased (Table 3). There were numerous correlations within the variables of interest, but these are not modeled in a way to show causation.

Table 6: Correlations between Changes in variables of interest for last half of season

Correlations

Change in:		Ferritin (3-2)	Performance	LBM	%BF	Caloric Intake 3
Ferritin (3-2)	Spearman r	1.000	-.083	-.327	.003	.511*
	Sig. (2-tailed)	.	.707	.118	.990	.021
	N	24	23	24	24	20
Performance	Spearman r		1.000	.091	.078	-.466*
	Sig. (2-tailed)		.	.671	.716	.038
	N		24	24	24	20
LBM	Spearman r			1.000	-.543**	-.112
	Sig. (2-tailed)			.	.005	.630
	N			25	25	21
%BF	Spearman r				1.000	-.026
	Sig. (2-tailed)				.	.911
	N				25	21
Caloric Intake 3	Spearman r					1.000
	Sig. (2-tailed)					.
	N					21

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Discussion:
The purpose of this study was to document iron status throughout the collegiate swimming season, while documenting diet, training and performance measures. The results of the current study suggest that serum ferritin levels do change throughout the season. There were significant differences in ferritin at the end of the season as compared with pre- and mid-season measures. Past studies have demonstrated that intense physical exercise negatively effects iron status [8]. Although the complete mechanisms are still unknown, previous studies have demonstrated and advocated that impairment comes from either changes in saturation levels of transferrin, inhibition of release of iron from its carrier, and/or a decrease in iron-containing oxidative enzymes [8, 13].

More recent literature has also suggested that hepcidin could be another mechanism for impairing iron status. A 2010 study (Karl et al.) [20] found that there was a relationship between iron status and hepcidin levels in female cadets during a 9-week training period. Hepcidin is a hormonal regulator of iron at the enterocyte level. It inhibits iron absorption in the enterocytes, as well as seizing it in macrophages, ultimately leading to a decrease in total body iron storage [23]. It has been suggested in previous studies that hepcidin is regulated through an inflammatory response cycle [10, 12, 20, 28]. When inflammation occurs, such as during intense, physical training, pro-inflammatory cytokines are released. This inflammation in turn stems the release of hepcidin, further preventing iron from being absorbed. The greater the inflammation that occurs, the greater the release of cytokines and hepcidin, leading to a greater decrease in the absorption of iron [10, 12, 20, 23, 28]. With little research on the relationship between hepcidin and iron status, especially in female athletes, this is a fertile area for future research to determine other mechanisms for decreased iron status.

One limitation of the present study was that it did not measure SF levels before the athletes started their workouts. The first measurement period occurred at the beginning of September, which was a month after the athletes had already started water and dry land training. The insignificant change seen between the first and second SF measurement periods could be related to inflammation levels. It is hypothesized that even by a month into training the athletes' bodies had already accumulated an inflammatory response significant enough to reduce the SF levels. This could explain why there was no change between the first and second ferritin measurements. The inflammatory response hypothesis would also explain why the SF levels significantly increased from the second to third measurements periods. Like most athletes, swimmers taper for their championship meets by reducing training volume and intensity. Mujika et al. [25] found that taper for competitive swimmers increased hemoglobin and hematocrit concentrations in the blood due to decreased hemolysis and net increase of erythrocytes, which ultimately led to an improved performance. Although this current study was unable to measure the intensity of the training, Figure 2 shows the volumes did decrease. Tapering in both the weight room and the water would decrease the intensity and amount the swimmers were working out, potentially decreasing inflammation. As the athletes began to taper for the Big Ten Swimming Championships between the second and third measurement periods, the intensity, volume, and duration of the workouts decreased. Theoretically, the inflammation accumulated in the body decreased, thus resulting in increased iron absorption as seen in

the increased SF levels. However, future research would need to be completed with measurements regarding inflammation (such as hepcidin) and with accurate baseline measurements obtained before training began in order to explore this hypothesis.

Another portion of the original hypotheses stated that iron status would increase with increasing supplementation and/or dietary modifications to increase iron intake. Although the present study did not find any strong correlations between supplemental compliance or dietary modifications and increased SF, there was a weak, positive relationship between both factors and SF indicating that supplementation/diet modifications have a positive effect on SF. With only 24 women in the study, it could be that the study was underpowered due to such high variability of ferritin in the group. Other studies have shown a positive relationship with supplementation and increased SF levels [5, 6, 13, 15].

The present study did not find a correlation between improved iron status and performance ($p=0.707$, $r=-0.083$), refuting the original hypothesis that increased ferritin would correlate with improved performance. However, another limitation to the present study was that there was no performance marker at the beginning of the measurements to determine changes from pre- to midseason. Other studies have shown varying results for iron status and its relationship to performance in aerobically based athletes. Similar to the present study, a 2004 study of Greek adolescent swimmers (Tsalis et al.) [34] and a 2006 study of collegiate female swimmers (Petersen et al.) [29] found no relationship between iron status and performance. Results for improved performance for athletes with low serum ferritin levels without anemia are controversial [13, 26]. However, there have been multiple other studies that have found that improved iron status, as measured by SF, did have a positive correlation to improved performance for endurance athletes, such as rowers, cross country runners, and swimmers [5, 10, 13, 15]. More controlled studies using the same markers would help tease out the circumstances that improve ferritin and performance.

It is well documented that individuals with low serum ferritin and hemoglobin levels, classifying as ID with anemia (IDA), have a decreased erythropoiesis and red blood cell production. This leads to a decreased oxygen-carrying capacity and overall decrease in performance. However, it is still debated whether athletes with just low SF levels (ID without anemia) have improved performance via these mechanisms by improving SF levels. Studies by Friedmann [13] Brownlie 2002 and 2004 [5-6] have all shown that improving SF levels, regardless of hematological levels, improves performance. Nonetheless, it is important to note that the 2002 Brownlie et al. [5] study measured performance increases in previously untrained women, not highly adapted athletes. The present study did not measure hemoglobin levels, and thus cannot determine if the athletes had ID or IDA. In order to better understand this mechanism in female swimmers, future research should include hematological measurements in order to determine hemoglobin levels and transferrin saturation levels as well as other markers of inflammation and training status.

Limitations:
As a pilot study, this study provided lots of data. However, there were several limitations to this study. First, even though the online food frequency questionnaires have been validated as being as reliable and easy to use as pencil tests [21], the dietary data for this study was not reliable. The graphical tests were taken three times during the course of the study, and asked participants questions regarding their dietary habits. The more accurately the test was filled out, the longer it took. The time it took each subject to complete the test was recorded. The questionnaire was supposed to take between 20-30 minutes, however, data revealed that some athletes took it in as little as less than 10 minutes. This would cause the results of the questionnaire to be extremely skewed, which was reflected in the low daily caloric intake for many of the subjects.

A second limitation to the study was the timing of the baseline measurements of serum ferritins. One of the goals of this study was to monitor the serum ferritin levels from the beginning of the season all the way through the end. However, by the time the first measurement had taken place, subjects had already been training for a month, and swimming at this collegiate level does not really provide a clear time where no training occurs. Therefore, a resting pre-season measurement was not obtained. Further studies should work towards having serum ferritin measurements taken before training has started.

A third limitation to this study was the aerobic capacity measurement system. Originally, the subject's maximum VO_2 were to be measured throughout the season. It was originally established that a submaximal bike test was to be in place. However, the protocol used during the first measurement period was extremely inaccurate, estimating VO_2 values that were extremely low or implausibly high. For the second measurement period the Astrand protocol was implemented, which produced less extreme results. However, many of the values produced were still not plausible. The submaximal test was not administered for the third measurement due to unreliable results. However, aerobic capacity data, such as a submaximal test, would be a great marker to have for future studies, especially for relating it to performance markers.

Conclusion:
This pilot study showed that ferritin levels changed throughout the year, but that performance and iron status (as measured by serum ferritin) were not related. However, future research should look further into reasons for why ferritin levels changed, such as inflammatory responses.

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Appendix 1

Ferritin and Iron

What does low ferritin mean?

- Ferritin is the storage form of iron in your body.
- When iron levels are low, the body has lower levels of red blood cells. This can lead to anemia in certain individuals.
- Low ferritin levels may also delay recovery from intense exercise.
- Iron is needed to form hemoglobin, a protein in healthy red blood cells that delivers oxygen from the blood.
- Iron is needed to form myoglobin, a protein that delivers oxygen in cells.

Ferritin Levels

- Very Low: <30 ng/mL
- Low: 30-50 ng/mL
- Good: >50 ng/mL

Signs of Anemia

- Fatigue
- Pale Skin
- Weakness
- Headache
- Dizziness or lightheadedness
- Cold hands and feet
- Irritability
- Brittle Nails
- Fast Heartbeat
- Cravings to chew ice
- Poor Appetite

Ferritin's Effect on Recovery

- Adequate iron is needed to create healthy red blood cells to deliver oxygen and remove wastes from muscle cells. Accumulation of these wastes can contribute to muscle pain.
- When skeletal muscle is damaged during exercise, myoglobin is released into the bloodstream and excreted. Adequate iron stores are needed to replenish lost myoglobin.

How do I Increase my Ferritin?

- The best way to increase ferritin stores is to increase iron intake and absorption from heme and non-heme food sources.
- Try to consume red meats 2-3 times a week.
- Eat high-iron vegetables in place of low-iron vegetables.
- If put on an iron supplement, take as prescribed.
- Avoid dairy and calcium supplements when taking supplements or during high-iron meals. Calcium can inhibit absorption of iron.

Food Sources of Iron

Heme Iron = Animal Sources

Readily Absorbed

- ☑ Lean Red Meet (especially beef)
- ☑ Dark Poultry
- ☑ Salmon
- ☑ Tuna
- ☑ Liver
- ☑ Oysters

Non-Heme Iron = Plant Sources

Less Readily Absorbed

- ☑ Iron Supplements
- ☑ Leafy Green Vegetables
  - Spinach
  - Kale
  - Broccoli
  - Collards
  - Asparagus
- ☑ Dried Beans
  - Lima Beans
  - Soybeans
  - Kidney Beans
- ☑ Dried Fruits
  - Prunes
  - Raisins
  - Apricots
- ☑ Whole Grains
  - Wheat
  - Millet
  - Oats
  - Brown Rice
- ☑ Iron-fortified breakfast cereals

Iron Supplements

- If your ferritin is low you may need to take an iron supplement.
- Different iron supplements are absorbed differently. Consult your team physician, dietitian, and athletic trainer to see what supplement is right for you.
- If you take a multi-vitamin containing iron, make sure it is USP verified.

Tips

- Vitamin C enhances the absorption of iron. Take your iron supplement with orange juice, or put citrus fruit on a leafy green salad.
- Calcium reduces the absorption of calcium. Avoid calcium supplements and excessive dairy in high iron-containing meals.
- Look for the highest percentage daily value of iron on food labels possible.

***Ferritin* is a storage protein for iron. When iron stores are low in the body, so is ferritin. Iron stores support your ability to do aerobic or endurance work. The way to improve your ferritin is to improve your iron intake and absorption.**

Food Sources of iron (resourced from http://www.nlm.nih.gov/medlineplus/ency/article/002422.htm)
The best food sources of iron include:

Heme iron, MFP factor, better absorbed	Non-heme iron, not as well absorbed
• Lean red meat (especially beef) • Oysters • Poultry, dark red meat • Salmon • Tuna • Liver	• Dried beans • Dried fruits • Eggs (especially egg yolks) • Iron-fortified cereals ○ Mini-wheats, Total • Whole grains

Reasonable amounts of iron are also found in lamb, pork, and shellfish.

Iron from vegetables, fruits, grains, and supplements are harder for the body to absorb. These sources include:

• Dried fruits
  ○ prunes
  ○ raisins
  ○ apricots
• Legumes
  ○ lima beans
  ○ soybeans
  ○ dried beans and peas
  ○ kidney beans
• Seeds
  ○ almonds
  ○ Brazil nuts
• Vegetables
  ○ broccoli
  ○ spinach
  ○ kale
  ○ collards
  ○ asparagus
  ○ dandelion greens
• Whole grains
  ○ wheat
  ○ millet
  ○ oats
  ○ brown rice
	Do: If you mix some lean meat, fish, or poultry with beans or dark leafy greens at a meal, you can improve absorption of vegetable sources of iron up to three times. Foods rich in vitamin C also increase iron absorption.
	Don't: Some foods reduce iron absorption. For example, commercial black or pekoe teas contain substances that bind to iron so it cannot be used by the body.
	
	Iron supplementation:
	• USP certified such as Nature Made multi
	  ○ Looking for 18 mg in a supp
	• Want a higher dosage, consider Feosol Bifera http://www.bifera.com

Iron is required to be listed on food labels and percentage based on 18 mg so looking for high percentage of DV.

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Appendix 2

Score your agreement for each statement where 7 means you strongly agree and a 1 means there is no relationship or agreement for you.

Fatigue Symptom Score Questionnaire

During the past week, I have found that:	Score						
1. My motivation is lower when I am fatigued.	1	2	3	4	5	6	7
2. Exercise brings on my fatigue.	1	2	3	4	5	6	7
3. I am easily fatigued.	1	2	3	4	5	6	7
4. Fatigue interferes with my physical functioning.	1	2	3	4	5	6	7
5. Fatigue causes frequent problems for me.	1	2	3	4	5	6	7
6. My fatigue prevents sustained physical functioning.	1	2	3	4	5	6	7
7. Fatigue interferes with carrying out certain duties and responsibilities.	1	2	3	4	5	6	7
8. Fatigue is among my three most disabling symptoms.	1	2	3	4	5	6	7
9. Fatigue interferes with my work, family, or social life.	1	2	3	4	5	6	7

Continued Athlete Menstrual and Fatigue/Compliance Questionnaire

1) How tired did you feel during and after practice before your ferritin level was checked? Please check one answer.
   - ❑ 1 very tired (profound, could not train)
   - ❑ 2
   - ❑ 3
   - ❑ 4
   - ❑ 5 not tired at all (absent, no fatigue)
2) Were you surprised by your last ferritin level? Please check one answer.
   - ❑ Yes
   - ❑ No
   - ❑ I was never given my result.
3) What were you told to do as a result of your level? Please check all that apply.
   - ❑ Nothing
   - ❑ Take an iron supplement, either over the counter or prescribed
   - ❑ Eat better
4) If you were told to take an iron supplement, how often are you taking it? Please check one answer.

- ❑ I was not told to take a supplement.
- ❑ Never
- ❑ Sometimes (between 1-3 days a week)
- ❑ Most days (between 4-6 days a week)
- ❑ Every day

5) Which measures have you done to try to increase iron in your diet? Please check all that apply
   - ❑ None
   - ❑ Eating more red meat
   - ❑ Eating more beans or other suggested plant sources of iron
   - ❑ Eating highly fortified (with iron) products such as cereals
   - ❑ Drinking orange juice when I eat food with iron in it
6) Do you feel your performance has improved? Please check one answer.
   - ❑ No
   - ❑ Yes, related to the above changes.
   - ❑ Yes, related to other factors. Please list:

     _____
     _____
7) How tired have you felt during and after practice in the past week? Please check one answer.
   - ❑ 1 very tired (profound, could not train)
   - ❑ 2
   - ❑ 3
   - ❑ 4
   - ❑ 5 not tired at all (absent, no fatigue)

8) Are you having regular periods, meaning a cycle once every 26-34 days?
   - ❑ No
   - ❑ Yes

9) If no to number 8, please explain how you are different.
   - Text response

10) How heavy do you feel your menstrual flow is most months?
   - ❑ Very heavy
   - ❑ Heavy
   - ❑ Average
   - ❑ Light
   - ❑ Very light

11) Are you on birth control pills?
   - ❑ Yes
   - ❑ No

12) Do you exercise outside of your sport training?
   - ❑ No
   - ❑ Yes

13) If yes, please tell us what activity and a weekly number of minutes spent in that activity?